



THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : 10/019,164 Confirmation No. 3977
Applicant : Benjamin J. Metcalf
Filed : December 20, 2001
TC/A.U. : 1645
Examiner : Patricia Ann Duffy
Docket No. : ACY33484-00
Customer No. : 25291

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.132

Sir:

I, Bruce A. Green, do hereby state and declare that:

1. I am a citizen of the United States, residing in New City, New York.
2. I am currently employed by Wyeth and have held the position of Research Fellow since January 2005. I obtained a B.A. from Rutgers College in 1976, and a Ph.D. from University of Iowa School of Medicine in 1980.
3. From 1983 to 1989, I was a staff scientist for Praxis Biologics working on *Haemophilus influenzae* vaccine antigens, specifically protein vaccines against nontypeable *H. influenzae* (NTHi). I was promoted to Manager of the Bacteriology Department in 1989 when Praxis was bought by American Cyanamid and served as Manager, then Associate Director of that group from 1989 to 1994. I continued on as Associate Director of the Bacteriology Department in Wyeth-Lederle Vaccines (now Wyeth Vaccines) after the merger of Lederle-Praxis with Wyeth. I directed research on NTHi and *Moraxella catarrhalis* vaccines and provided scientific input on many other vaccine projects. I managed the department of up to 15 scientists and have published

extensively over the years on NTHi antigens, particularly the P4 and P6 proteins. I am internationally regarded as an expert in the field of vaccines against NTHi and *M. catarrhalis*, and have written several reviews on the subject in peer-reviewed journals. I thus have considerable experience in this field and am very competent to provide the statements contained in this declaration. A copy of my CV is attached.

4. I have read and am familiar with the present application and the Office Actions dated November 25, 2003, May 13, 2004 and January 25, 2005. I am particularly familiar with the primary reference—Anilionis et al. (WO 90/02557) -- cited by the Examiner, as the subject matter of the present application came out of the same laboratory, Praxis Biologics, which is now Wyeth Vaccines.

5. The following experiment was designed to examine the relative expression level of lipidated rP6 protein from arabinose- and *lac*-promoted plasmid constructs in the same *E. coli* strain.

Experimental Procedure:

6. *Escherichia coli* strain BLR was used as the host strain for expression analysis. Purified plasmid pPX162 (which contains the gene encoding the lipidated P6 protein under control of the *lac* promoter on a high copy number plasmid) was transformed into *E. coli* BLR and transformants selected on Hy-Soy agar plates containing carbenicillin (carb; 100 µg/ml). *E. coli* BLR(pPX4020) (which contains the gene encoding the lipidated P6 protein under control of the arabinose promoter) was also used in these experiments and selected using chloramphenicol (cam; 30 µg/ml).

7. Overnight cultures of each bacterial strain grown in liquid Hy-Soy medium containing either carb or cam were diluted 1:40 in HySoy containing the appropriate antibiotic and grown to an OD₆₀₀ ~1.0. Strains BLR(pPX4020) and BLR(pPX162) were induced with 0.2% arabinose and 1mM IPTG, respectively, and grown for 3 additional hours. Whole cell lysates in SDS-PAGE cracking buffer containing β-mercaptoethanol were made by boiling at ~100 °C for 10 minutes. Lysate equivalent to approximately 10⁷ cfu were loaded onto each lane of a 4-20% Tris-Glycine gel and electrophoresed in SDS-PAGE buffer. Gels were stained with Coomassie brilliant blue using standard

methods. For Western analyses, mAb G125-6TX, which is specific for the *Haemophilus influenzae* P6 protein, was used at 1:5000 followed by goat α -mouse Ig-biotin @ 1:3000.

Results:

8. SDS-PAGE analysis was used for evaluation of the relative amounts of rP6 expressed by the two induced cultures and presence of rP6 in uninduced cultures. The Coomassie stained gel analysis of the uninduced and induced cultures (Fig. 1A) shows that the induced bacteria containing each plasmid expressed the P6 protein. However, the level of P6 expressed by the BLR(pPX4020) strain is substantially increased relative to the level of P6 expressed by the pPX162 containing strain as shown by the dark rP6 band at ~16 kDa.

9. Western blot analysis was performed to confirm the identity of the induced protein band as the rP6 protein and to detect any expression of the rP6 protein in the uninduced versus induced cultures. The amount of protein loaded into the lanes of the gel used for the Western blot analysis was overloaded as compared to the amount used for normal Western blot analysis to increase sensitivity for low amounts of rP6 in the uninduced cultures and thus is not suitable to show differences between the larger amounts of rP6 in the induced cultures. This is due to the amounts of rP6 in these cultures being outside of the linear range of the Western blot as performed here. The Western blot shown in Figure 1B clearly shows that the rP6 protein is expressed by both pPX162 and pPX4020. Additionally, extremely low levels of rP6 (barely detectable by Western blot) are expressed in the uninduced cultures of the pPX4020 containing strain, as is typical for the tightly regulated arabinose promoter system. In contrast, the pPX162 uninduced culture contains much larger amounts of detectable rP6 protein, demonstrating that the *lac* promoter in the pPX162 is not tightly regulated, allowing significant "leak through" expression in the absence of any inducer.

Conclusions:

10. Based on my years of experience working with the *H. influenzae* P6 protein, and my extensive experience developing expression systems for this protein in *E. coli*, I conclude that the level of rP6 protein expressed by plasmid pPX4020 which contains the gene encoding lipidated P6 under control of the arabinose promoter is substantially greater than that expressed by the pPX162 plasmid which contains the same gene

under control of the *lac* promoter. Our laboratory tried for years to greatly increase expression levels of lipidated P6 protein in *E. coli* and we were unsuccessful, as described in our publication from 1990 (Green et al., Infect. Immun. 58(10):3272-3278). Another laboratory also tried and failed to overexpress lipidated P6 protein in *E. coli* and also had to make non-lipidated rP6 many years later (Yang, Y. P., et al., 1997, Vaccine 15:976-87).

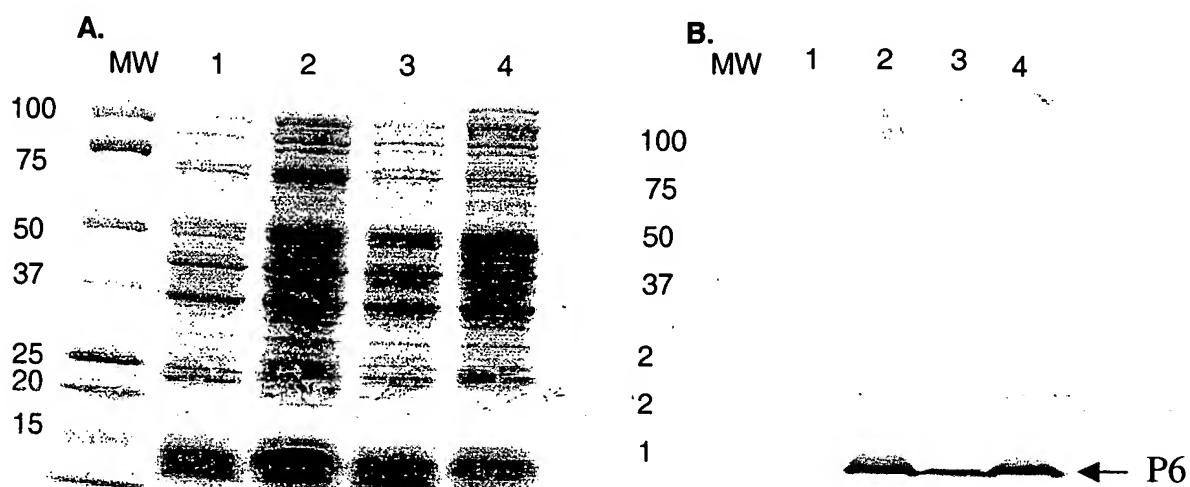
11. I also conclude from the Western blot analysis of the cultures that the arabinose promoted construct (pPX4020) is tightly regulated and, in the absence of the inducer, expressing levels of rP6 so low that even Western blot can barely detect a band of rP6. This is in direct contrast to the *lac*-promoted rP6 gene contained in plasmid pPX162 which expresses easily detectable amounts of rP6 even in the absence of the IPTG inducer. This is another example of the "leakiness" of the *lac* promoter as described throughout the literature.

12. The undersigned declarant declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize any patent issuing from the present application.

Signed this 25th day of July, 2005


Bruce A. Green

Figure 1. SDS-PAGE analysis of whole cell lysates from BLR(pPX4020) & BLR (pPX162). A) Coomassie Stain; B) Western Blot analysis. Lanes: BLR(pPX4020) uninduced (lane 1) and induced with arabinose (lane 2); BLR(pPX162) uninduced (lane 3) and induced with IPTG (lane 4).





9/15/2003

BRUCE ARTHUR GREEN
25 Verona Court
New City, New York 10956

Telephone Numbers: (845) 602-2754 (W)
(845) 602-5536 (Fax)
(845) 639-5388 (H)
email: lavgreen@rpa.net

Birthdate:

August 13, 1954

Personal Information:

Married, 2 children

Educational Background:

1976 B.A. Rutgers College, Rutgers University, New Brunswick, NJ
Major: Microbiology

1980 Ph.D. University of Iowa, Iowa City, IA
Major: Pathogenic Bacteriology

Dissertation Title: Immunogenicity of proteins present on ribosomes
isolated from type 14 *Streptococcus pyogenes*.

Relevant Experience:

2005-Present. Research Fellow, Associate Director, Discovery-to-Development Transition Bacterial Vaccines, and Bacterial Virulence Identification Group

2003-2004. Associate Director, Discovery-to-Development Transition Bacterial Vaccines, and Bacterial Virulence Identification Group

2001-Discovery research leader in meetings with FDA's Center for Biologics Evaluation and Research (CBER) concerning intranasal vaccine administration

1998-2003. Assoc. Director, Bacteriology Research, Wyeth Vaccines. Manager of 11 people, including 4 PhDs. Responsible for administration, departmental budget, and personnel reviews

1995- Present: Ad Hoc Reviewer for NIH on R01 Grants (Average of 2 per year)

1994-1998. Associate Research Fellow & Manager, Bacteriology Research, Wyeth-Lederle Vaccines and Pediatrics. Supervisor of Bacteriology department of 15 people, 4 PhDs.

1989-1994. Manager, Bacteriology Research, Lederle-Praxis Biologicals. Responsible for continuing research on nontypable *H. influenzae* project and supervision of Bacteriology department of 14 people, 4 PhDs.

1986-89. Senior Research Scientist, Bacteriology Research, Praxis Biologics. Responsible for part of continuing research on OMPs of *H. influenzae* and research on *Bordetella pertussis*. Principle investigator on NIH SBIR grants, phase I and II, and NIAID R 01 grant.

1985-86. Acting Manager, Bacteriology Research, Praxis Biologics. Responsible for Bacteriology research personnel and research on outer membrane proteins of *Haemophilus influenzae*.

1984-85. Manager, GMP Production of PRP vaccine, Praxis Biologics, Rochester, NY. Responsible for GMP production of vaccine (licensed in 1985) and inspection of manufacturing facility by FDA.

1983-84. Research Scientist, Praxis Biologics, Rochester, NY working on *Shigella flexnerii* pathogenesis.

1980-83. Postdoctoral fellow with Dr. R.K. Holmes, Dept. of Microbiology, Uniformed Services University of the Health Sciences researching *Vibrio cholerae* genetics and cholera toxin.

1976-80. Graduate research on ribosomal vaccine of *S. pyogenes* with Dr. W. Johnson, Univ. of Iowa.

1975-76. Undergraduate research with Dr. B. Koft, Rutgers University on *in vivo* and *in vitro* biosynthesis of pyridoxine.

CURRENT RESPONSIBILITIES:

Associate Director, Discovery-to-Development Transition, Bacteriology

Associate Director, Bacterial Virulence Identification Group

Oversight and Consulting on GLP/GMP Production of Clinical Material for Toxicity/Pathology and Phase I Clinical Studies

Review of External Scientific Collaborations

INVITED LECTURES:

Invited speaker on Intranasal vaccination against nontypeable *H. influenzae*, 3rd International Symposium on Tonsils and Mucosal Barriers of the Upper Airways, Wakayama, Japan, April 9, 2003

Invited speaker on Modern Development of Vaccines, 1st International Greek Biotechnology Forum, Athens, Greece, June 10-13, 2004

GRANTS FUNDED:

Principal Investigator on NIH SBIR Grant: An *Haemophilus* OMP as a Vaccine Candidate, \$50,000, 1987.

Principal Investigator on NIH SBIR Grant: *Haemophilus* PAL as a Vaccine Candidate for Otitis Media, \$250,000, 1989.

Principal Investigator on NIH R 01 Grant: *Haemophilus* OMPs as Vaccine Candidates for Otitis Media, \$230,000, 1990.

PATENTS ISSUED:

U.S. Patent # 5,098,997 Vaccines for *Haemophilus influenzae*

U.S. Patent # 5,110,908 *Haemophilus influenzae* peptides and proteins

U.S. Patent # 5,108,744 Vaccines for *Haemophilus influenzae*

U.S. Patent # 5,196,338 Recombinant vectors for *Haemophilus influenzae* peptides and proteins.

U.S. Patent # 5,601,831 Vaccines for Nontypable *Haemophilus influenzae*

U.S. Patent # 5,643,725 Sequence and analysis of LKP pili structural genes and the LKP Pili Operon of Nontypable *Haemophilus influenzae*

U.S. Patent #5,780,601 Method for purification of protein "E" from *Haemophilus influenzae*

U.S. Patent # 5,955,580 Vaccines for nontypable *Haemophilus influenzae*

U.S. Patent # 5,968,769 Sequence and analysis of LKP pili structural genes and the LKP Pili Operon of nontypable *Haemophilus influenzae*

U.S. Patent # 6,420,134 B1 Vaccines for nontypeable *Haemophilus influenzae*.

MEMBERSHIPS:

American Society for Microbiology
International Society for Vaccines.

PUBLICATIONS:

1. T. Wu, J. Chen, T. F. Murphy, **B. A. Green**, and X.-X. Gu. 2005. Investigation of nontypeable *Haemophilus influenzae* outer membrane protein P6 as a new carrier for lipooligosaccharide-based conjugate vaccine. (Submitted for Publication).

2. **Green, B.A.**, E. Baranyi, T. J. Reilly, A. Howell, A. L. Smith, and G.W. Zlotnick. 2005. Site Directed Mutation of the *Haemophilus influenzae* P4 Lipoprotein Affects Its Immunogenicity and Elicitation of Bactericidal Antibodies. Infect. Immun. (in press).
3. Hotomi, M., M. Suzumoto, K. Yamauchi, **B. A. Green**, G. Zlotnick, D. S. Billal, Y. Ikeda, S. Tamura, K. Fujihara and N. Yamanaka. 2005. A recombinant P4 protein of *Haemophilus influenzae* induces specific immune responses biologically active against nasopharyngeal colonization in mice after intranasal immunization. Vaccine. 23(10):1294-1300.
4. **Green, B.A.**, Y. Zhang, A.W. Masi, V. Barniak, M. Wetherell, R.P. Smith, M.S. Reddy, and D.Z. Zhu. 2005. PppA, a surface exposed protein of *Streptococcus pneumoniae*, elicits cross-reactive antibodies that reduce colonization in a murine intranasal immunization and challenge model. Infect. Immun. 73:(2)981-989.
5. Giebink, G. S., L. O. Bakaletz, S. J. Barenkamp, **B. Green**, X.-X. Gu, T. Heikkinen, M. Hotomi, P. Karma, Y. Kurono, J. M. Kyd, T. F. Murphy, P. L. Ogra, J. A. Patel, and S. I. Pelton. 2005. Recent Advances in Otitis Media. 8. Vaccines. Ann. Otol. Rhinol. Laryngol. Suppl. 114:S86-103.
6. Liu, D. F., K.W. Mason, M. Mastro, M. Pazirandeh, D. Cutter, D.L. Fink, J. W. St Geme, 3rd, D. Zhu, and **B. A. Green**. 2004. The C-Terminal Fragment of the Internal 110-Kilodalton Passenger Domain of the Hap Protein of Nontypeable *Haemophilus influenzae* Is a Potential Vaccine Candidate. Infect Immun 72:6961-8.
7. Mason, K. W., D. Zhu, C. A. Scheuer, J. C. McMichael, G. W. Zlotnick, and **B. A. Green**. 2004. Reduction of nasal colonization of nontypeable *Haemophilus influenzae* following intranasal immunization with rLP4/rLP6/UspA2 proteins combined with aqueous formulation of RC529. Vaccine 22:3449-3456.
8. McMichael, J.C. and **B.A. Green**. 2003. Vaccines for *Moraxella catarrhalis* and nontypeable *Haemophilus influenzae*. Curr. Opin. Invest. Drugs 4(8): 953-958..
9. Fink, DL, A.Z. Buscher, **B.A. Green**, P. Fernsten, and J.W. St. Geme, III. 2003. The *Haemophilus influenzae* Hap autotransporter mediates microcolony formation and adherence to epithelial cells and extracellular matrix via binding epitopes in the C-terminal end of the passenger domain. Cellular Micro. 5(3):175-186.

10. Cutter, D., K. W. Mason, A. P. Howell, D. L. Fink, **B. A. Green**, and I. J. St Geme. 2002. Immunization with *Haemophilus influenzae* Hap Adhesin Protects against Nasopharyngeal Colonization in Experimental Mice. *J Infect Dis* **186**:1115-21.
11. Hotomi, M., N. Yamanaka, J. Shimada, M. Suzumoto, Y. Ikeda, A. Sakai, J. Arai, and **B. Green**. 2002. Intranasal immunization with recombinant outer membrane protein P6 induces specific immune responses against nontypeable *Haemophilus influenzae*. *Int. J. Pediatr Otorhinolaryngol* **65**:109-16.
12. **Green, B.A.**, and S.M. Baker. 2002. Recent advances and novel strategies in vaccine development. *Curr. Opinion Micro.* **5**(5):483-488.
13. Fink, D. L., **B. A. Green**, et al. 2002. The *Haemophilus influenzae* Hap Autotransporter Binds to Fibronectin, Laminin, and Collagen IV. *Infect Immun* **70**(9): 4902-7.
14. Bakaletz LO, Barenkamp SJ, Eskola J, **Green B**, Gu XX, Harada T, Heikkinen T, Karma P, Klein JO, Kurono Y, et al. 2002. **Recent advances in otitis media. 7. Vaccine.** *Ann Otol Rhinol Laryngol Suppl*, **188**:82-94.
15. Kemmer, G., T. Reilly, J. Schmidt-Brauns, G.W. Zlotnick, **B.A. Green**, M.J. Fiske, M. Herbert, A. Kraiß, Stefan Schlör, A. Smith and J. Reidl. 2001. NadN and e(P4) are essential for the utilization of NAD and NMN but not NR in *Haemophilus influenzae*. *J. Bacteriol.* **183**(13):3974-3981.
16. Reilly, T.J., **B.A. Green**, G.W. Zlotnick, and A.L. Smith. 2001. Contribution of the DDDD motif of *H. influenzae* e (P4) to Phosphomonoesterase activity and Heme transport. *FEBS Letters* **494**:19-23.
17. Zhang, Y., A. Masi, V. Barniak, K. Mountzouros, M. Hostetter, and **B.A. Green**. 2001. Recombinant PhpA protein, a unique histidine-motif containing protein from *Streptococcus pneumoniae*, protects mice against intranasal pneumococcal challenge. *Infect. Immun.* **69**: 3827-3826
18. P.W. Tebbey, C.A. Scheuer, J.A. Peek, D. Zhu, N.A. LaPierre, **B.A. Green**, E.D. Phillips, A.R. Ibraghimov, J.H. Eldridge and G.E. Hancock. 2000. Effective mucosal immunization against respiratory syncytial virus using purified F protein and a genetically detoxified cholera holotoxin, CT-E29H. *Vaccine* **18**:2723-2734.
19. Zagursky, R.J., P. Ooi, M.J. Fiske, K.F. Jones, and **B.A. Green**. 2000. Identification of a *Haemophilus influenzae* 5'-nucleotidase: Cloning of the *nucA* gene and immunogenicity of the NucA protein. *Infect. Immun.* **68**(5):2525-2534.
20. **Green, B. A.**, S. B. Olmsted, M. Vazquez, G. Quigley-Reape, and G. W. Zlotnick. 1998. A Clinical Vaccine Formulation for *Haemophilus influenzae* Otitis

Media Containing Recombinant P4 and P6 Outer Membrane Proteins. In *Recent Advances in Otitis Media: Proceedings of the Fourth Extraordinary International Symposium*, M. Tos, Thomsen, J, and Balle, V., eds. Kugler Publications, Amsterdam, The Netherlands. p.355-362.

21. **Green, B.A.**, E.S. Meredith, L. Edwards, and K.F. Jones. 1994. "Nontypeable *Haemophilus influenzae* lipooligosaccharide conjugates as vaccine candidates against NTHi." In *Vaccines 94: Modern approaches to new vaccines including prevention of AIDS*, p. 125- 129., Norrby ed. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.
22. **Green, B.A.**, W.J. Doyle, and J.L. Cowell. 1994. "Chinchilla model of experimental otitis media for study of nontypable *Haemophilus influenzae* vaccine efficacy." In *Methods in Enzymology: Bacterial Pathogenesis, Part A. Identification and regulation of virulence factors.*, p. 59-68. Bavoil ed. Academic Press. San Diego, CA.
23. **Green, B.A.**, M.E. Vazquez, G.W. Zlotnick, G. Quigley-Reape, J.D. Swarts, I. Green, J.L. Cowell, C.D. Bluestone, and W.J. Doyle. 1993. Evaluation of mixtures of purified *Haemophilus influenzae* (Hi) outer membrane proteins to protect against challenge with nontypable Hi in the chinchilla otitis media model. *Infect. Immun.* **61**: 1950-57.
24. Deich, R.A., A. Anilionis, J.P. Fulginiti, T. Quinn-Dey, G.W. Zlotnick, and **B.A. Green**. 1993. Recombinant hybrids of *Haemophilus influenzae* (Hi) outer membrane lipoproteins PCP and PAL as vaccine candidates against nontypable H. influenzae disease. *Vaccine Research* **2**: 31-39.
25. Rustici, A., M. Velucchi, R. Faggioni, M. Sironi, P. Ghezzi, S. Quataert, **B. Green**, and M. Porro. 1993. Molecular mapping and detoxification of the lipid A binding site by synthetic peptides. *Science* **259**: 361-365.
26. **Green, B.A.**, and R.A. Deich. 1991. Bacterial lipoproteins as vaccines candidates and patent considerations. *Current Opinion in Therapeutic Patents* **1**: 669-680.
27. **Green, B.A.**, J.E. Farley, T. Quinn-Dey, R.A. Deich, and G.W. Zlotnick. 1991. The e (P4) outer membrane protein of *Haemophilus influenzae*: Biologic activity of anti-e serum and cloning and sequencing of the structural gene. *Infect. Immun.* **59**: 3191-3198.
28. Deich, R.A., A. Anilionis, J. Fulginiti, B.J. Metcalf, S.A. Quataert, T. Quinn-Dey, G.W. Zlotnick, and **B.A. Green**. 1990. Antigenic conservation of the 15,000-dalton outer membrane lipoprotein PCP of *Haemophilus influenzae* and biologic activity of anti-PCP antisera. *Infect. Immun.* **58**: 3388-3393.

29. **Green, B.A.**, B.J. Metcalf, T. Quinn-Dey, D.H. Kirkley, S.A. Quataert, and R.A. Deich. 1990. A recombinant non-fatty acylated form of the Hi-PAL (P6) protein of *Haemophilus influenzae* elicits biologically active antibody against both nontypable and type b *H. influenzae*. *Infect. Immun.* **58**: 3272-3278.
30. Deich, R.A., B.J. Metcalf, C.W. Finn, J.E. Farley, and **B.A. Green**. 1988. Cloning of genes encoding a 15,000-dalton peptidoglycan-associated outer membrane lipoprotein and an antigenically related 15,000-dalton protein from *Haemophilus influenzae*. *J. Bacteriol.* **170**: 489-498.
31. **Green, B.A.**, T. Quinn-Dey, and G.W. Zlotnick. 1987. Biologic activities of antibody to a peptidoglycan-associated lipoprotein of *Haemophilus influenzae* against multiple clinical isolates of *H. influenzae* type b. *Infect. Immun.* **55**: 2878-2883.
32. Deich, R.A., and **B.A. Green**. 1987. Mobilization of *Haemophilus influenzae* chromosomal markers by an *Escherichia coli* F' factor. *J. Bacteriol.* **159**: 1905-1910.
33. **Green, B.A.**, J.W. Newland, and R.K. Holmes. 1986. "Genetic Mapping of biotype determinants and molecular cloning of the polymixin B resistance gene(s) of *Vibrio cholerae* El Tor." *In Advances in Research on Cholera and Related Diarrheas.*, 227-232. S. Kuwahara ed. KTK Scientific Publishers. Tokyo, Japan.
34. Holmes, R.K., **B.A. Green**, E.M. Twiddy, R.J. Neill, and W.T. Ruyechan. 1986. "Genetic and biochemical studies of a new heat-labile enterotoxin of *Escherichia coli* that activates adenylate cyclase." *In Advances in Research on Cholera and Related Diarrheas.*, 221-226. S. Kuwahara ed. KTK Scientific Publishers. Tokyo, Japan.
35. Newland, J.W., **B.A. Green**, and R.K. Holmes. 1985. Cloning of extracellular DNase and construction of a DNase negative strain of *Vibrio cholerae*. *Infect. Immun.* **47**: 691-696.
36. Newland, J.W., **B.A. Green**, and R.K. Holmes. 1984. Transposon-mediated mutagenesis and recombination in *Vibrio cholerae*. *Infect. Immun.* **45**: 428-432.
37. **Green, B.A.**, J.W. Newland, and R.K. Holmes. 1983. Mapping of chromosomal genes that determine the El Tor biotype of *Vibrio cholerae*. *Infect. Immun.* **42**: 924-929.
38. **Green, B.A.**, R.J. Neill, W.T. Rucheyn, and R.K. Holmes. 1983. Evidence that a new enterotoxin of *Escherichia coli* which activates adenylate cyclase in eucaryotic target cells is not plasmid-mediated. *Infect. Immun.* **41**: 383-390.

39. **Green, B.A.**, and W. Johnson. 1980. Immunogenicity of ribosomes from enzymatically lysed *Streptococcus pyogenes*. Infect. Immun. **27**: 424-430.